

FURTHER STUDIES OF TESTIS CYTOLOGY IN MICE  
IRRADIATED WITH 2450-MHz MICROWAVES



Male mice, 10 to 12 weeks old, were housed overnight in an anechoic chamber and exposed to 2450 MHz CW radiation for 0, 2, or 16 h. Up to 10 mice were caged singly in polypropylene cages with food and water and irradiated in a multi-unit cage array located within the 1.8 m diameter quiet zone. Cages were spaced by  $3\lambda$  to minimise interaction. Power density incident on each cage position depended on its angle relative to the central axis and interaction with neighbouring objects.

In one experiment two groups of 70 B6C3F1 mice were exposed for 4 days, 16 h/day, and were sacrificed at intervals up to 10 weeks for sperm count and percent abnormal sperm. The two groups averaged 20 and 32 mW/cm<sup>2</sup>. We reported last year that immersion of the hindquarters in a waterbath at 40°C for 2 h caused a marked depression of the sperm count and elevation of abnormal sperm from 2 to above 30 per cent from 1½ to 2½ weeks later. Neither of the two microwave-irradiated groups showed any effect on sperm count or percent abnormal sperm.

Another experiment was designed to meet the criticism that our failure to find microwave effects on the testis, as reported last year and this, might be attributed to our choice of the hybrid B6C3F1 mouse which could be particularly resistant to microwave damage. We therefore compared B6C3F1 with three inbred strains, C57Bl/6B, C3H/HeB, Balb/c, and a random-bred line, Swiss Webster. Four groups of 10 mice were exposed for 0, 2, or 16 h irradiation in the anechoic chamber (average power density 26 mW/cm<sup>2</sup>) or the hindquarters in a waterbath at 40°C for 2 h. Five were killed immediately for assay for dark-stained cells in testis, sperm count, and percent abnormal sperm; five were killed 2 weeks later for sperm count and percent abnormal sperm. This time interval was chosen because there was a large effect of heat at this interval (see experiment above). An effect of the heating was demonstrated on all strains, but none showed an effect of microwaves.

Tests of the reproductive effectiveness of male mice irradiated in the anechoic chamber for 4 days (16 h/day) at an average power density of 39 mW/cm<sup>2</sup> are under way and preliminary results will be available.

## SUMMARY

The testis is frequently cited as a critical organ for limiting microwave exposure, but there have been very few studies of the physiology of the testis in microwave-irradiated animals. Since the testis is known to be sensitive to elevation of its temperature we are engaging in a comparison of its response to heat and microwaves.

The requirements for complete specification of the physical conditions of microwave exposure conflict with those for physiological experimentation and the following notes indicate the compromises adopted in our work.

(1) We believe both acute and chronic exposure should be investigated and that for the latter animal restraint is not acceptable.

(2) We irradiate for up to 16 h/day in far field with the animals housed singly in polypropylene cages. The cages contain a standard amount of bedding, 4 food cubes, and a small water bottle. They are arranged to minimize second-order interactions. Control animals are caged identically in the anechoic chamber or animal room. The temperature is held at  $23 \pm 1^\circ\text{C}$  and relative humidity is 50% or less.

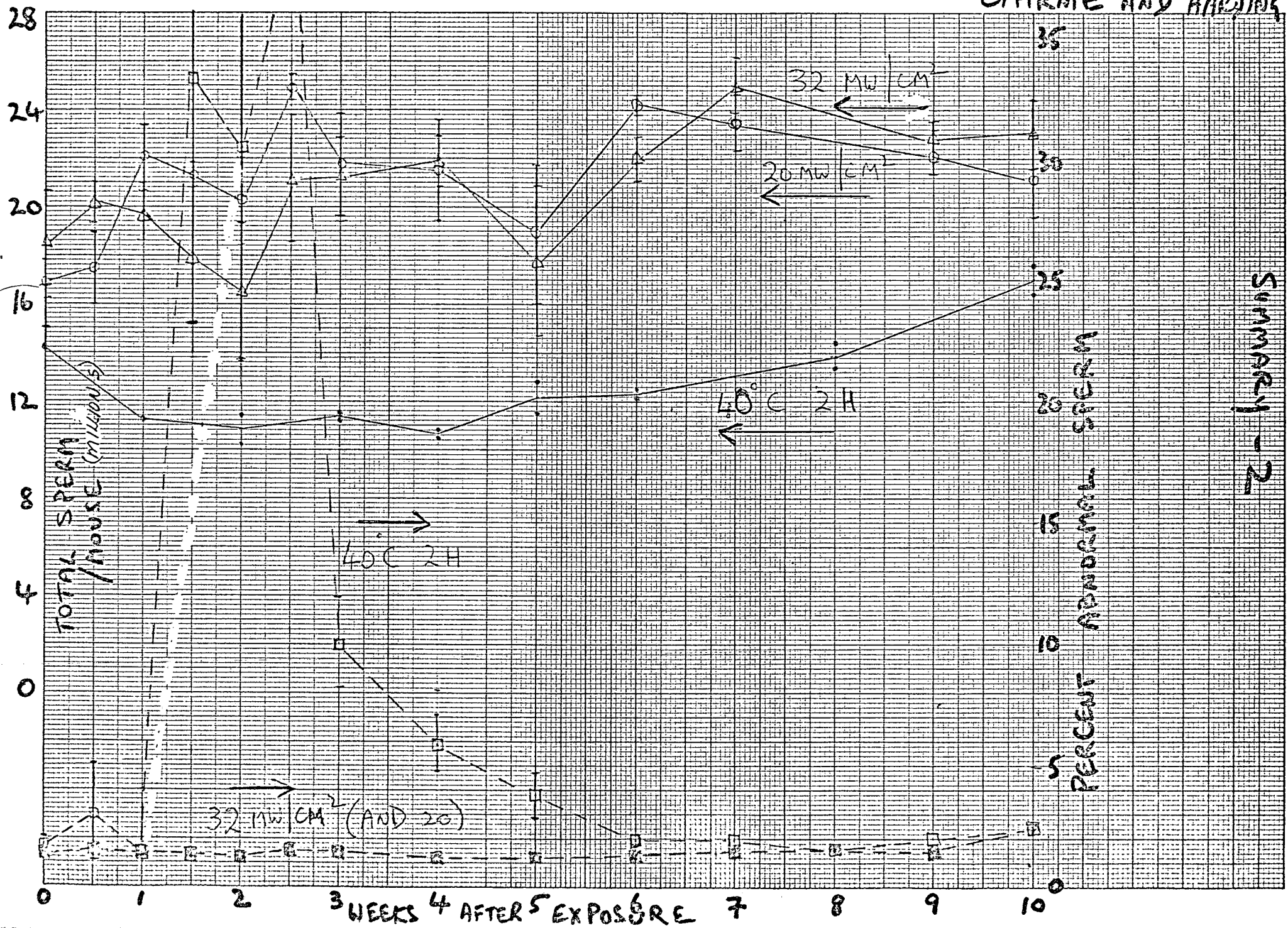
(3) Dosimetry is accomplished by measuring the exposure in each cage position on replacing that cage by a Holaday Model 1500 probe. No elevation of testis temperature has been detected despite careful investigation. Preliminary measurements of E field in the testis for various mouse orientations have been made and will be reported.

(4) We regard temperature and E field in the testis as more meaningful parameters than whole-body SAR, and exposure as more practical for defining hazards in the eventual extrapolation to possible harmful effects in man.

FIRST EXPERIMENT The results of this experiment, which was described in the abstract, are shown in the accompanying graph.  $40^\circ\text{C}$  for 2 h, but not  $38^\circ$  or lower for 4 h, caused a fall in sperm count (the content of both epididymides) and an elevation of the percentage of sperm with abnormalities. Exposure to microwaves for 4 days (16 h/day, 20 or  $32 \text{ mW/cm}^2$ ) had no effect.

SECOND EXPERIMENT The following table gives the results on sperm count and percent dark-stained cells. We have developed the dark-stained cell assay for measuring the amount of cell damage in testis due to heat or similar insults. The scoring of sperm abnormalities is not yet complete. As indicated in the abstract, the four other strains studied responded very similarly to the B6C3F1 strain to both heat and microwaves.

CAIRNIE AND HARDING



Summary - 2

Mouse Strain	Sacrifice at 0 weeks								Sacrifice at 2 weeks			
	Percent dark cells				Sperm Count (MILLIONS)				Sperm Count (MILLIONS)			
	Exposure (h)				Exposure (h)				Exposure (h)			
	Microwaves		40°C		Microwaves		40°C		Microwaves		40°C	
0	2	16	2	0	2	16	2	0	2	16	2	
B6C3F1	2.2 +0.8	0.9 +0.4	1.2 +0.2	7.6 +1.8	30.7 +1.1	26.8 +1.6	23.7 +0.9	23.6 +1.2	26.0 +1.0	30.1 +1.2	27.2 +2.8	6.8 +0.9
C57B1/6B	1.0 +0.4	0.8 +0.02	0.5 +0.2	6.7 +0.7	13.7 +1.6	16.1 +0.4	15.5 +0.9	13.4 +1.5	15.8 +0.7	16.6 +1.3	17.9 +0.9	9.0 +1.7
C3H/HeB	1.0 +0.7	1.0 +0.07	1.8 +0.5	20.0 +3.2	12.1 +1.3	12.7 +1.3	10.5 +0.6	16.7 +1.2	14.4 +1.9	9.7 +1.2	12.6 +1.0	6.1 +1.3
Balb/c	2.0 +0.3	1.4 +0.5	1.3 +0.4	13.0 +1.3	12.9 +0.4	12.2 +0.9	13.6 +0.9	12.1 +0.6	13.1 +0.4	12.3 +1.7	14.8 +1.4	6.1 +0.6
Swiss Webster	0.6 +0.4	1.0 +0.4	1.3 +1.0	6.8 +1.3	22.4 +1.5	22.6 +2.7	26.4 +2.8	21.0 +0.6	27.5 +1.5	22.5 +1.4	27.5 +3.9	6.9 +1.2

Table Each figure given is the mean + SE mean for a group of 5. For the percent dark-stained cells assay 500 cells were scored from each animal. For the sperm count the cells were diluted and 4 aliquots making up  $10^{-4}$  of the volume counted for each animal.